UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/[illegible]	[illegible]	[illegible]	.2481.0790-0

FINNEGAN HENDERSON FARABOW
GARRETT AND DUNNER
1300 I STREET NW
WASHINGTON DC 20005-3315

EXAMINER	
SAUD, S	
ART UNIT	PAPER NUMBER
1801	41

DATE MAILED: 09/03/97

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

☒ Responsive to communication(s) filed on 06 June 1997

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), ~~or thirty days~~, ~~whichever is longer,~~ from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 21-23, 25-27, 31-32 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 21-23, 25-27, 31-32 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of Reference Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

– SEE OFFICE ACTION ON THE FOLLOWING PAGES –

★ U.S GPO: 1996-410-238/40050

DETAILED ACTION

1. Claim 31 has been amended and claim 32 has been added. Claims 21-23, 25-27 and 31-32 are currently pending and under consideration in the instant application.

2. Any objection or rejection of record which is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Response to Arguments

4. Applicant's arguments filed 06 June 1997 have been fully considered but they are not persuasive.

Claim Rejections - 35 USC § 112

5. Claims 21 and 22 (and dependent claim 23) are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for "expressing in a bacterium a DNA molecule encoding a fusion protein", does not reasonably provide enablement for "expressing as part of a fusion protein in a bacterium a DNA molecule encoding a mini-proinsulin". The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

This language is found in step (a) of claims 21 and 22, however, the specification does not contemplate or teach making a construct which consists of a fusion protein and a DNA molecule. This interpretation of the claims is because the limitation of step (a) requires expressing a DNA molecule as part of a fusion protein. It seems clear that Applicant intended to claim expression of a DNA molecule encoding a fusion protein in a bacteria, but the claims do not reflect this at the present time. Amendment of the claims to language which finds support in the instant specification (such as language used in claims 25-26) would obviate this ground of rejection. (There are no arguments regarding this rejection because it is a new ground of rejection.)

6. Claim 32 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 32 requires all of the steps of the method be performed in one vessel. Support for this claim is said to be found at page 2, lines 7-20. However, the instant specification states "The immediate conversion of the compound of the formula I into insulin in a 'one-pot reaction' is particularly advantageous." The compound of formula I is the miniproinsulin of the formula B(1-30)-Arg-A(1-21). This statement does not provide support for the invention as it now appears in claim 32 which encompasses the making of the fusion protein in a single vessel and the addition of cyanogen bromide to that vessel, incubating with sodium tetrathionate in the same vessel, addition

of trypsin and carboxypeptidase in the same vessel and ending with precipitation of insulin in the same vessel. Furthermore, it appears that the separation of the fusion protein from the bacteria would be a necessary step in the method prior to the addition of cyanogen bromide.

7. Claim 32 is rejected under 35 U.S.C. 112, first paragraph, as based on a disclosure which is not enabling. A method step which separates the fusion protein from the bacteria in step (a) of this claim is critical or essential to the practice of the invention, but not included in the claim(s) is not enabled by the disclosure. *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976). The method of claim 32 is a method "wherein all of (a) through (e) are performed in one vessel." However, it appears that the isolation of the fusion protein from the bacteria which produces it is essential to the practice of the invention. This is because the next step in the method is the addition of cyanogen bromide, which if added to a bacterial preparation, would cause such a jumble of proteins that it would not predictably result in the ability to effectively isolate any particular protein from the composition created thereby, absent clear and convincing evidence to the contrary. (This is a new ground of rejection necessitated by the addition of this claim in the most recent amendment.)

8. Claims 21-23, 25-27 and 31 remain rejected under 35 U.S.C. 112, first paragraph, and newly added claim 32 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one

skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention for the reasons of record in paper #38 as applied to claims 21-23, 25-27, 31.

Applicant argues that the specification need not describe the claimed invention using the identical words found in the claims in order to satisfy the requirements of 35 U.S.C. 112, first paragraph. This argument is not found persuasive, because although the this statement is true, the inherent property of a single embodiment or example in the specification does not provide support for a general idea of a negative limitation. As stated previously, the mere absence of a positive limitation does not provide basis for a negative limitation. Applicant states that the issue is one of whether the concept expressed by the phrase "under conditions where no crystals are formed" is present in the specification as filed. It is the Examiner's position that this concept does not find support in a single example wherein the conditions are such that no crystals are formed and does not provide a basis for claims including the negative limitation "under conditions where no crystals are formed". Applicant also cites MPEP § 2163.07(a) for support of the addition of the negative limitation. However, claiming a "function, theory or advantage" of a device with inherent properties is not the same as including a negative limitation which is present in a single example of the instant application and then claiming that this single embodiment provides basis for the general concept of conditions under which no crystals are formed. Applicant further states that MPEP § 2173.05(I) is not pertinent is not persuasive and still applies to the issue at hand in this rejection and the rejection is maintained.

It is noted that Applicant is correct in that the Examiner confused miniproinsulin of the formula B(1-30)-Arg-A(1-21) with the mono-Arg-insulin of formula I (see page 2 of the instant specification). Therefore, for the sake of clarity, the rejection will be repeated in its entirety below and arguments pertinent to the rejection will be addressed. Claims 21, 23 and 25 are directed to preparation of mon-Arg-insulin and claims 22, 26, 27, 31 and 32 are directed to the preparation of insulin.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claim 21 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Markussen et al. (U.S. Pat. No. 4,916,212) or Markussen et al. (EPO 163,529) either in view of Goeddel et al. (EPO 055,945), Grau (U.S. Pat. No. 4,801,684) and Grau (U.S. Pat. No. 4,639,332) essentially as applied to the claims in the prior Office actions (paper #'s 32 and 29).

Markussen et al. ('212) discloses insulin precursors of the form B(1-29)-X_n-Y-A(1-21). "X" is a peptide chain with n amino acids, "n" is an integer from 0 to 33, and "Y" is Lys or Arg. X is preferably selected from the group consisting of Ala, Ser, and Thr. A preferred embodiment is B(1-29)-Ser-Lys-A(1-21). This precursor protein is a single peptide chain. This precursor is converted to human insulin by derivatization and treatment with trypsin. (See '212 at column 2, line 65, through column 3, line 46; Examples 11, 13, and 16; and claims.) Fusion proteins and their cleavage from the precursor are disclosed. (See column 5, lines 11-20.) DNA sequence

encoding the insulin precursor, expression vectors, transformed cells, and recombinant methods of production in yeast (as well as E. coli holding plasmids encoding the desired insulin precursors) are also disclosed and claimed. Markussen et al. ('529) teaches essentially the same invention. (See pages 5-6, 8; Table 1; Example 11, page 26; Example 13, page 29; Example 16, page 30; claims.) The Markussen references do not specifically teach the preparation of mono-Arg-insulin which includes the use of trypsin as cleavage agent for generation of mono-Arg-insulin.

The miniproinsulin of the instant application is directed to a single peptide chain of the formula B(1-30)-Arg-A(1-21). The amino acid at position 30 in native human insulin is Thr. This position is equivalent to the "X" of Markussen et al.

Goeddel et al. teach producing recombinant fusion proteins of insulin precursors to another protein and cleaving them. The reference further teaches making a fusion protein with an insulin variant in which the C chain of insulin contains only six amino acids. (See page 6, line 19 through page 8, line 2; abstract; claims; pages 26-27.) Goeddel et al. also teaches production in E. coli. With regard to fusion proteins, it is noted that E. coli has long been used to produce desirable precursors to insulin and that fusion proteins are often used for small peptides.

Grau ('684) teaches using trypsin and carboxypeptidase B simultaneously to produce mature insulin from proinsulin. (See column 5, lines 49-59.)

Grau ('332) teaches that treatment of proinsulin with trypsin alone gives intermediates with an arginine at B31. This insulin-Arg^{B31}-OH derivative is stable to further tryptic degradation. Enzymes having both tryptic and carboxypeptidase B activity are required to produce insulin.

(See column 1, lines 1-32; column 2, lines 10-12.) The intermediate disclosed by Grau ('332) is the mono-Arg-insulin of formula II in the instant claims.

Markussen et al. suggest the claimed miniproinsulin precursor, DNA sequences encoding it, vectors, host cells and process for preparation where "X" is Thr, "n" is 1, and "Y" is Arg. This is a very similar structure to the preferred embodiment B(1-29)-Ser-Lys-A(1-21). The claimed generic formula of the prior art encompasses Applicant's claimed composition in method step (a). It would have been obvious to one of ordinary skill in the art at the time the invention was made to use this particular embodiment as suggested by Markussen et al. for the production of mono-Arg-insulin as taught by Grau ('332 and '684) because mono-Arg-insulin is exceptionally stable to further tryptic degradation (column 2, lines 10-12 of Grau '332) and makes this species of miniproinsulin an ideal and obvious choice for use in the preparation of mono-Arg-insulin. Furthermore, it would have been obvious to make fusion proteins as taught by Goeddel et al. using the insulin precursor, DNA sequences, and vectors taught by either Markussen et al. reference for the production of the mono-Arg-insulin of Grau and to cleave the fusion protein to release the desired protein as taught by Goeddel et al. One would have been motivated by the known benefits of producing small peptides as fusion proteins in bacterial and yeast hosts and the success with another insulin variant in which the C chain is shortened and because the usefulness of fusion proteins is suggested by Markussen et al. It would have been obvious to prepare mono-Arg-insulin by expressing a DNA molecule encoding miniproinsulin in bacteria as taught and suggested by Markussen et al. and cleaving this compound with trypsin as taught by Grau ('332

and '684) to produce mono-Arg-insulin. One would have been motivated to produce this stable intermediate of insulin for further treatment with carboxypeptidase B to produce insulin for treating diabetes. The limitation of "under conditions where no crystals are formed" is met in that the methods of Markussen demonstrate that the processes take place in an aqueous buffer (acetic acid) with the isolation of the protein via precipitation with acetone (see column 18, lines 42-68 of Markussen '212). Finally, it would have been obvious to one of ordinary skill in the art at the time the invention was made to precipitate the resulting mono-Arg-insulin for the isolation of the desired product as taught by Markussen '212 (column 18, lines 42-68). Therefore, the invention as a whole would have been *prima facie* obvious at the time it was made, absent clear and convincing evidence to the contrary.

11. Claim 25 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Markussen et al. (U.S. Pat. No. 4,916,212) or Markussen et al. (EPO 163,529) either in view of Goeddel et al. (EPO 055,945), Grau (U.S. Pat. No. 4,801,684) and Grau (U.S. Pat. No. 4,639,332) essentially as applied to the claims in the prior Office actions (paper #'s 32 and 29), further in view of Mai et al. as applied to claim 21 above.

The disclosures of Markussen et al., Goeddel et al. and Grau are as described above. These references do not specifically teach the bridging member Met-Ile-Glu-Gly-Arg of step (a) in the claim.

Mai et al. teach that it would have been well known in the art to use common cleavage sites in fusion proteins. The reference teaches that cyanogen bromide cleaves after the amino acid

Met and that factor Xa cleaves after the tetrapeptide Ile-Glu-Gly-Arg. (See column 3, line 14, through column 4, line 35, especially column 3, line 67, through column 4, line 1; and column 9, lines 7-19.)

It would have been obvious to make the miniproinsulin of Markussen et al. as a fusion protein using the cleavable sequence Met-Ile-Glu-Gly-Arg as taught in Mai et al. for the production of mono-Arg-insulin of Grau. Markussen et al. suggest making fusion proteins that can be cleaved as does Goeddel et al. The recited sequence of the claim includes cleavage sites for cyanogen bromide and factor Xa that would have been commonly used in fusion proteins and would have been well known to the skilled artisan. One would have been motivated to make a fusion protein for the reasons taught by Markussen et al., Goeddel et al., and Mai et al. and to use this construct in the method of making mono-Arg-insulin of Grau for the advantages taught therein, absent clear and convincing evidence to the contrary. Therefore, the invention as a whole would have been *prima facie* obvious at the time it was made, absent clear and convincing evidence to the contrary.

12. Claims 22 and 23 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Markussen et al. (U.S. Pat. No. 4,916,212) or Markussen et al. (EPO 163,529) either in view of Goeddel et al. (EPO 055,945), Grau (U.S. Pat. No. 4,801,684) and Grau (U.S. Pat. No. 4,639,332) essentially as applied to the claims in the prior Office actions (paper #'s 32 and 29).

The disclosures of Markussen et al., Goeddel et al. and Grau are as described above. Markussen et al. do not teach a method of making insulin using both trypsin and carboxypeptidase B to convert miniproinsulin to mono-Arg-insulin and then to insulin.

It would have been obvious to use both trypsin and carboxypeptidase B to convert the miniproinsulin of Markussen et al. (having the formula B(1-30)-Arg-A(1-21)) first to mono-Arg-insulin and then to insulin. Grau ('332) teaches that mono-Arg-insulin can be formed by trypsin cleavage and that this form is resistant to further tryptic degradation and Grau ('684) teaches that the combination of trypsin and carboxypeptidase B together can convert proinsulin to insulin. One would have been motivated to use both trypsin and carboxypeptidase B in order to produce insulin from the precursor of Markussen et al. for treating diabetes. One would also have a reasonable expectation of success in obtaining insulin from the miniproinsulin precursor of Markussen et al. because the miniproinsulin having the formula B(1-30)-Arg-A(1-21) is very similar to the preferred embodiment of Markussen et al. and because this miniproinsulin would provide for the stable mono-Arg-insulin of Grau in the process of making insulin. The limitation of "under conditions where no crystals are formed" is met in that the methods of Markussen demonstrate that the processes take place in an aqueous buffer (acetic acid) with the isolation of the protein via precipitation with acetone (see column 18, lines 42-68 of Markussen '212). It also would have been obvious to one of ordinary skill in the art at the time the invention was made to precipitate the resulting mono-Arg-insulin for the isolation of the desired product as taught by Markussen '212 (column 18, lines 42-68). Finally, claim 23 requires that the trypsin and

carboxypeptidase B be added in the same vessel; Grau '684 teaches the process wherein trypsin and carboxypeptidase B were added together (i.e. in the same vessel) and resulted in the production of mature insulin from proinsulin (see column 5, lines 57-59). Therefore, the invention as a whole would have been *prima facie* obvious at the time it was made, absent clear and convincing evidence to the contrary.

13. Claims 26-27 and 31 stand rejected (and newly added claim 32 is rejected) under 35 U.S.C. § 103(a) as being unpatentable over Markussen et al. (U.S. Pat. No. 4,916,212) or Markussen et al. (EPO 163,529) either in view of Goeddel et al. (EPO 055,945), Mai et al., Grau (U.S. Pat. No. 4,801,684) and Grau (U.S. Pat. No. 4,639,332) essentially as applied to the claims in the prior Office actions (paper #'s 32 and 29) and claims 22-23 above.

The disclosures of Markussen et al., Goeddel et al., Grau and Mai et al. are as described above. None of these references teach the method of the claims in its entirety. Claims 26-27 and 31-32 are directed to methods using both trypsin and carboxypeptidase B to convert miniproinsulin to mono-Arg-insulin and then to insulin, including a bridging member Met-Ile-Glu-Gly-Arg between the fusion protein and the miniproinsulin. Claims 31-32 also include the limitation of "without formation of substantial amounts of insulin Des-B30.

It would have been obvious to use both trypsin and carboxypeptidase B to convert the miniproinsulin of Markussen et al. (having the formula B(1-30)-Arg-A(1-21)) first to mono-Arg-insulin and then to insulin. Grau ('332) teaches that mono-Arg-insulin can be formed by trypsin cleavage and that this form is resistant to further tryptic degradation and Grau ('684) teaches that

the combination of trypsin and carboxypeptidase B together can convert proinsulin to insulin. One would have been motivated to use both trypsin and carboxypeptidase B in order to produce insulin from the precursor of Markussen et al. for treating diabetes. One would also have a reasonable expectation of success in obtaining insulin from the miniproinsulin precursor of Markussen et al. because the miniproinsulin having the formula B(1-30)-Arg-A(1-21) is very similar to the preferred embodiment of Markussen et al. and because this miniproinsulin would provide for the stable mono-Arg-insulin of Grau in the process of making insulin. It also would have been obvious to make the miniproinsulin of Markussen et al. as a fusion protein using the cleavable sequence Met-Ile-Glu-Gly-Arg as taught in Mai et al. for the production of mono-Arg-insulin of Grau. Markussen et al. suggest making fusion proteins that can be cleaved as does Goeddel et al. The recited sequence of the claim includes cleavage sites for cyanogen bromide and factor Xa that would have been commonly used in fusion proteins and would have been well known to the skilled artisan. One would have been motivated to make a fusion protein for the reasons taught by Markussen et al., Goeddel et al., and Mai et al. and to use this construct in the method of making mono-Arg-insulin of Grau for the advantages taught therein, absent clear and convincing evidence to the contrary. The limitation of "under conditions where no crystals are formed" is met in that the methods of Markussen demonstrate that the processes take place in an aqueous buffer (acetic acid) with the isolation of the protein via precipitation with acetone (see column 18, lines 42-68 of Markussen '212). It also would have been obvious to one of ordinary skill in the art at the time the invention was made to precipitate the resulting mono-Arg-insulin for

the isolation of the desired product as taught by Markussen '212 (column 18, lines 42-68). Claim 27 requires that the trypsin and carboxypeptidase B be added in the same vessel; Grau '684 teaches the process wherein trypsin and carboxypeptidase B were added together (i.e. in the same vessel) and resulted in the production of mature insulin from proinsulin (see column 5, lines 57-59). Lastly, claims 31 and 32 recite "without formation of substantial amounts of insulin Des-B30. One of ordinary skill in the art would not expect the methods of Markussen et al. and Grau for the production of insulin from miniproinsulin and mono-Arg-insulin to result in the formation of substantial amounts of Des-B30 insulin, nor are there any steps in the instant claims which would distinguish from the prior art in resulting in different amounts of Des-B30 insulin, absent clear and convincing evidence to the contrary. Therefore, the invention as a whole would have been *prima facie* obvious at the time it was made, absent clear and convincing evidence to the contrary.

Response to Arguments

In response to Applicant's request in footnote #1 at page 7, this limitation has been addressed in the rejection of claims 31 and 32 above.

Applicant argues that the prior art of record would not have suggested using the species of Grau in the Markussen process. Applicant also further clarifies the difference between mono-Arg-insulin and mininproinsulin of the formula B(1-30)-Arg-A(1-21). As stated before the grounds of rejection under 103, the Examiner did confuse the two compounds, and therefore, the

rejections have been restated and clarified. As to the suggestion of using mono-Arg-insulin of Grau in the process of Markussen, Grau itself provides the motivation for using mono-Arg-insulin because Grau teaches the exceptional stability of mono-Arg-insulin to further tryptic degradation. This teaching provides the motivation and suggestion to use the specific embodiment of B(1-30)-Arg-A(1-21) of Markussen et al., as well as the similarity of this embodiment to the admitted preferred embodiment of Markussen et al. (i.e. B(1-29)-Ser-Lys-A(1-21)). One would have a reasonable expectation of success in using this specific embodiment for the production of insulin as taught by Markussen et al. or for the production of mono-Arg-insulin.

Applicant argues, at page 9 of the response, that "those skilled in the art would not have expected trypsin to cleave at the C-terminus of a bridging Arg residue in the single chain 'precursor' to generate the two chain, mature form of insulin" from the teachings of Markussen et al. This is not found persuasive because Markussen et al. is not relied upon for the teaching of using trypsin for cleavage of miniproinsulin to mono-Arg-insulin; Grau '332 teaches the use of trypsin for the production of mono-Arg-insulin (see column 3-4, Example 1). In response to applicant's arguments against the references individually, one cannot show non-obviousness by attacking references individually where the rejections are based on combinations of references. *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicant argues that Thim et al. teach away from using trypsin to cleave the miniproinsulin into mono-Arg-insulin or insulin. This is not persuasive because Grau '332 specifically teaches using trypsin for this purpose as mentioned above.

Applicant next argues that there is no suggestion for using the claimed miniproinsulin B(1-30)-Arg-A(1-21) in any method. This point has been addressed in the rejections above. Applicant argues that there are a very large number of species which could be arrived at from Markussen's generic formula. However, in evaluating the formula in light of the preferred embodiments of Markussen et al. and the suggested mono-Arg-insulin of Grau, the specific embodiment of B(1-30)-Arg-A(1-21) would have been *prima facie* obvious to one of ordinary skill in the art. This is because B(1-30)-Arg-A(1-21) is the Markussen et al. formula wherein X=Thr (which is the naturally occurring amino acid found at position B30 and a conservative amino acid substitution for the preferred Ser of Markussen et al.), n=1, m=1 (a preferred embodiment of Markussen et al.; see column 3, lines 12-18), and Y=Arg (which is a conservative amino acid substitution for the preferred Lys of Markussen et al.). Applicant argues that based on *Baird*, "the Examiner has provided no reasons why one would have selected the miniproinsulin as the starting material to produce insulin, especially give the large number of possible compounds included within Markussen's generic formula" (at page 11 of response). This argument is not found persuasive and was properly addressed in paper #32, including reasons and explanation. Again as pointed out in the grounds of rejection, the miniproinsulin of the claims is an obvious variant of the preferred embodiment B(1-29)-Ser-Lys-A(1-21) of Markussen et al. It is an

obvious variant because the substitution of Thr for Ser and Arg for Lys are conservative amino acid substitutions and included in the possible embodiments of Markussen et al. and would be expected to function in a similar manner. Furthermore, the desirability of the mono-Arg-insulin of Grau, which has the formula of B(1-30)-Arg-A(1-21) wherein the B and A chains of insulin are separate and have the normal cysteine bonds of native insulin makes the miniproinsulin species B(1-30)-Arg-A(1-21) obvious because the cleavage of the miniproinsulin B(1-30)-Arg-A(1-21) with trypsin would provide mono-Arg-insulin as taught by Grau and the cleavage of miniproinsulin B(1-30)-Arg-A(1-21) with trypsin and carboxypeptidase B as taught by Grau would provide insulin, both of which are useful and desirable.

Applicant asserts that Grau '684 "dealt with processes for obtaining insulin precursors rather than processes for obtaining insulin from mono-Arg-insulin" (emphasis omitted; see page 11 of response) and that Grau describes a natural porcine proinsulin and not the miniproinsulin of the instant claims. This argument is not found to be persuasive because in response to applicant's arguments against the references individually, one cannot show non-obviousness by attacking references individually where the rejections are based on combinations of references. *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Grau '684 teaches that trypsin and carboxypeptidase B can be added simultaneously and Markussen et al. teach the miniproinsulin of the instant claims. Grau '332 teaches the addition of trypsin results in mono-Arg-insulin and enzymes having both tryptic and carboxypeptidase B activity are required to produce insulin. (See column 1, lines 1-32; column 2,

lines 10-12.) Therefore, the combination of references makes obvious the steps of adding trypsin and carboxypeptidase B together for the conversion of miniproinsulin to insulin, absent clear and convincing evidence to the contrary.

Applicant's arguments at page 12 are not persuasive because they appear to be a hypothetical situation. Based on the teachings of Grau, one would have been motivated to use the trypsin and carboxypeptidase B in producing insulin from miniproinsulin, absent clear and convincing evidence to the contrary. It is understood that every method has possible pitfalls, but the knowledge of potential pitfalls or possibilities does not teach away from an invention especially in light of the specific teaching taught in Grau. Applicant's arguments bridging pages 12-13 are also based on potential things that could go wrong in the method. However, as stated in the rejections above, Grau is not relied upon for the removal of the bridging member. Mai et al. is relied upon for the bridging member and trypsin is not required for the cleavage of the bridging member from the miniproinsulin (cyanogen bromide cleaves after the amino acid Met and that factor Xa cleaves after the tetrapeptide Ile-Glu-Gly-Arg). Applicant's methods use the words "comprising" which means that additional steps may be included and does not preclude the use of a different enzyme for the cleavage of the bridging member.

Conclusion

14. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Thursday from 8AM to 4PM. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Walsh, can be reached on (703) 308-2957. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4242. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Communications via Internet e-mail regarding this application, other than those under 35 U.S.C. 132 or which otherwise require a signature, may be used by the applicant and should be addressed to [**stephen.walsh@uspto.gov**].

All Internet e-mail communications will be made of record in the application file. PTO employees do not engage in Internet communications where there exists a possibility that sensitive information could be identified or exchanged unless the record includes a properly signed express waiver of the confidentiality requirements of 35 U.S.C. 122. This is more clearly set forth in the Interim Internet Usage Policy published in the Official Gazette of the Patent and Trademark on February 25, 1997 at 1195 OG 89.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Christine Saoud, Ph.D.
September 1, 1997
CA

JOHN ULM
PRIMARY EXAMINER
GROUP 1800